```
cgctcttacc tagtagaggt tgagtgaatt tcttgacttg tttctcctat tggtgtatct 60
cttaaaatat taaattcaaa atcaaagtat atattttaca atg aag tct tct ttc   115
                                          Met Lys Ser Ser Phe
                                            1               5

ccc aag ttt gta ttt tct aca ttt gct att ttc cct ttg tct atg att   163
Pro Lys Phe Val Phe Ser Thr Phe Ala Ile Phe Pro Leu Ser Met Ile
                  10                  15                  20

gct acc gag aca gtt ttg gat tca agt gcg agt ttc gat ggg aat aaa   211
Ala Thr Glu Thr Val Leu Asp Ser Ser Ala Ser Phe Asp Gly Asn Lys
              25                  30                  35

aat ggt aat ttt tca gtt cgt gag agt cag gaa gat gct gga act acc   259
Asn Gly Asn Phe Ser Val Arg Glu Ser Gln Glu Asp Ala Gly Thr Thr
          40                  45                  50

tac cta ttt aag gga aat gtc act cta gaa aat att cct gga aca ggc   307
Tyr Leu Phe Lys Gly Asn Val Thr Leu Glu Asn Ile Pro Gly Thr Gly
      55                  60                  65

aca gca atc aca aaa agc tgt ttt aac aac act aag ggc gat ttg act   355
Thr Ala Ile Thr Lys Ser Cys Phe Asn Asn Thr Lys Gly Asp Leu Thr
  70                  75                  80                  85
```

FIG. 1A

```
ttc aca ggt aac ggg aac tct cta ttg ttc caa acg gtg gat gca ggg   403
Phe Thr Gly Asn Gly Asn Ser Leu Leu Phe Gln Thr Val Asp Ala Gly
                  90                  95                 100

act gta gca ggg gct gct gtt aac agc agc gtg gta gat aaa tct acc   451
Thr Val Ala Gly Ala Ala Val Asn Ser Ser Val Val Asp Lys Ser Thr
             105                 110                 115

acg ttt ata ggg ttt tct tcg cta tct ttt att gcg tct cct gga agt   499
Thr Phe Ile Gly Phe Ser Ser Leu Ser Phe Ile Ala Ser Pro Gly Ser
         120                 125                 130

tcg ata act acc ggc aaa gga gcc gtt agc tgc tct acg ggt agc ttg   547
Ser Ile Thr Thr Gly Lys Gly Ala Val Ser Cys Ser Thr Gly Ser Leu
     135                 140                 145

agt ttg aca aaa aat gtc agt ttg ctc ttc agc aaa aac ttt tca acg   595
Ser Leu Thr Lys Asn Val Ser Leu Leu Phe Ser Lys Asn Phe Ser Thr
150                 155                 160                 165

gat aat ggc ggt gct atc acc gca aaa act ctt tca tta aca ggg act   643
Asp Asn Gly Gly Ala Ile Thr Ala Lys Thr Leu Ser Leu Thr Gly Thr
                 170                 175                 180

aca atg tca gct ctg ttt tct gaa aat acc tcc tca aag aaa ggc gga   691
Thr Met Ser Ala Leu Phe Ser Glu Asn Thr Ser Ser Lys Lys Gly Gly
             185                 190                 195
```

FIG. 1B

```
gcc att cag act tcc gat gcc ctt acc att act gga aac caa ggg gaa   739
Ala Ile Gln Thr Ser Asp Ala Leu Thr Ile Thr Gly Asn Gln Gly Glu
        200                 205                 210

gtc tct ttt tct gac aat act tct tcg gat tct gga gct gca att ttt   787
Val Ser Phe Ser Asp Asn Thr Ser Ser Asp Ser Gly Ala Ala Ile Phe
    215                 220                 225

aca gaa gcc tcg gtg act att tct aat aat gct aaa gtt tcc ttt att   835
Thr Glu Ala Ser Val Thr Ile Ser Asn Asn Ala Lys Val Ser Phe Ile
230                 235                 240                 245

gac aat aag gtc aca gga gcg agc tcc tca aca acg ggg gat atg tca   883
Asp Asn Lys Val Thr Gly Ala Ser Ser Ser Thr Thr Gly Asp Met Ser
                250                 255                 260

gga ggt gct atc tgt gct tat aaa act agt aca gat act aag gtc acc   931
Gly Gly Ala Ile Cys Ala Tyr Lys Thr Ser Thr Asp Thr Lys Val Thr
            265                 270                 275

ctc act gga aat cag atg tta ctc ttc agc aac aat aca tcg aca aca   979
Leu Thr Gly Asn Gln Met Leu Leu Phe Ser Asn Asn Thr Ser Thr Thr
        280                 285                 290

gcg gga gga gct atc tat gtg aaa aag ctc gaa ctg gct tcc gga gga   1027
Ala Gly Gly Ala Ile Tyr Val Lys Lys Leu Glu Leu Ala Ser Gly Gly
    295                 300                 305
```

FIG. 1C

```
ctt acc cta ttc agt aga aat agt gtc aat gga ggt aca gct cct aaa   1075
Leu Thr Leu Phe Ser Arg Asn Ser Val Asn Gly Gly Thr Ala Pro Lys
310                 315                 320                 325

ggt gga gcc ata gct atc gaa gat agt ggg gaa ttg agt tta tcc gcc   1123
Gly Gly Ala Ile Ala Ile Glu Asp Ser Gly Glu Leu Ser Leu Ser Ala
                330                 335                 340

gat agt ggt gac att gtc ttt tta ggg aat aca gtc act tct act act   1171
Asp Ser Gly Asp Ile Val Phe Leu Gly Asn Thr Val Thr Ser Thr Thr
            345                 350                 355

cct ggg acg aat aga agt agt atc gac tta gga acg agt gca aag atg   1219
Pro Gly Thr Asn Arg Ser Ser Ile Asp Leu Gly Thr Ser Ala Lys Met
        360                 365                 370

aca gct ttg cgt tct gct gct ggt aga gcc atc tac ttc tat gat ccc   1267
Thr Ala Leu Arg Ser Ala Ala Gly Arg Ala Ile Tyr Phe Tyr Asp Pro
    375                 380                 385

ata act aca gga tca tcc aca aca gtt aca gat gtc tta aaa gtt aat   1315
Ile Thr Thr Gly Ser Ser Thr Thr Val Thr Asp Val Leu Lys Val Asn
390                 395                 400                 405

gag act ccg gca gat tct gca cta caa tat aca ggg aac atc atc ttc   1363
Glu Thr Pro Ala Asp Ser Ala Leu Gln Tyr Thr Gly Asn Ile Ile Phe
                410                 415                 420
```

FIG. 1D

```
aca gga gaa aag tta tca gag aca gag gcc gca gat tct aaa aat ctt   1411
Thr Gly Glu Lys Leu Ser Glu Thr Glu Ala Ala Asp Ser Lys Asn Leu
            425                 430                 435

act tcg aag cta cta cag cct gta act ctt tca gga ggt act cta tct   1459
Thr Ser Lys Leu Leu Gln Pro Val Thr Leu Ser Gly Gly Thr Leu Ser
        440                 445                 450

tta aaa cat gga gtg act ctg cag act cag gca ttc act caa cag gca   1507
Leu Lys His Gly Val Thr Leu Gln Thr Gln Ala Phe Thr Gln Gln Ala
    455                 460                 465

gat tct cgt ctc gaa atg gac gta gga act act cta gaa cct gct gat   1555
Asp Ser Arg Leu Glu Met Asp Val Gly Thr Thr Leu Glu Pro Ala Asp
470                 475                 480                 485

act agc acc ata aac aat ttg gtc att aac atc agt tct ata gac ggt   1603
Thr Ser Thr Ile Asn Asn Leu Val Ile Asn Ile Ser Ser Ile Asp Gly
                490                 495                 500

gca aag aag gca aaa ata gaa acc aaa gct acg tca aaa aat ctg act   1651
Ala Lys Lys Ala Lys Ile Glu Thr Lys Ala Thr Ser Lys Asn Leu Thr
            505                 510                 515

tta tct gga acc atc act tta ttg gac ccg acg ggc acg ttt tat gaa   1699
Leu Ser Gly Thr Ile Thr Leu Leu Asp Pro Thr Gly Thr Phe Tyr Glu
        520                 525                 530
```

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FIG. 1E

```
aat cat agt tta aga aat cct cag tcc tac gac atc tta gag ctc aaa   1747
Asn His Ser Leu Arg Asn Pro Gln Ser Tyr Asp Ile Leu Glu Leu Lys
    535                 540                 545

gct tct gga act gta aca agc acc gca gtg act cca gat cct ata atg   1795
Ala Ser Gly Thr Val Thr Ser Thr Ala Val Thr Pro Asp Pro Ile Met
550                 555                 560                 565

ggt gag aaa ttc cat tac ggc tat cag gga act tgg ggc cca att gtt   1843
Gly Glu Lys Phe His Tyr Gly Tyr Gln Gly Thr Trp Gly Pro Ile Val
                570                 575                 580

tgg ggg aca ggg gct tct acg act gca acc ttc aac tgg act aaa act   1891
Trp Gly Thr Gly Ala Ser Thr Thr Ala Thr Phe Asn Trp Thr Lys Thr
            585                 590                 595

ggc tat att cct aat ccc gag cgt atc ggc tct tta gtc cct aat agc   1939
Gly Tyr Ile Pro Asn Pro Glu Arg Ile Gly Ser Leu Val Pro Asn Ser
        600                 605                 610

tta tgg aat gca ttt ata gat att agc tct ctc cat tat ctt atg gag   1987
Leu Trp Asn Ala Phe Ile Asp Ile Ser Ser Leu His Tyr Leu Met Glu
    615                 620                 625

act gca aac gaa ggg ttg cag gga gac cgt gct ttt tgg tgt gct gga   2035
Thr Ala Asn Glu Gly Leu Gln Gly Asp Arg Ala Phe Trp Cys Ala Gly
630                 635                 640                 645
```

FIG. 1F

```
tta tct aac ttc ttc cat aag gat agt aca aaa aca cga cgc ggg ttt   2083
Leu Ser Asn Phe Phe His Lys Asp Ser Thr Lys Thr Arg Arg Gly Phe
                650                 655                 660

cgc cat ttg agt ggc ggt tat gtc ata gga gga aac cta cat act tgt   2131
Arg His Leu Ser Gly Gly Tyr Val Ile Gly Gly Asn Leu His Thr Cys
            665                 670                 675

tca gat aag att ctt agt gct gca ttt tgt cag ctc ttt gga aga gat   2179
Ser Asp Lys Ile Leu Ser Ala Ala Phe Cys Gln Leu Phe Gly Arg Asp
        680                 685                 690

aga gac tac ttt gta gct aag aat caa ggt aca gtc tac gga gga act   2227
Arg Asp Tyr Phe Val Ala Lys Asn Gln Gly Thr Val Tyr Gly Gly Thr
    695                 700                 705

ctc tat tac cag cac aac gaa acc tat atc tct ctt cct tgc aaa cta   2275
Leu Tyr Tyr Gln His Asn Glu Thr Tyr Ile Ser Leu Pro Cys Lys Leu
710                 715                 720                 725

cgg cct tgt tcg ttg tct tat gtt cct aca gag att cct gtt ctc ttt   2323
Arg Pro Cys Ser Leu Ser Tyr Val Pro Thr Glu Ile Pro Val Leu Phe
                730                 735                 740

tca gga aac ctt agc tac acc cat acg gat aac gat ctg aaa acc aag   2371
Ser Gly Asn Leu Ser Tyr Thr His Thr Asp Asn Asp Leu Lys Thr Lys
            745                 750                 755
```

FIG. 1G

```
tat aca aca tat cct act gtt aaa gga agc tgg ggg aat gat agt ttc   2419
Tyr Thr Thr Tyr Pro Thr Val Lys Gly Ser Trp Gly Asn Asp Ser Phe
        760                 765                 770

gct tta gaa ttc ggt gga aga gct ccg att tgc tta gat gaa agt gct   2467
Ala Leu Glu Phe Gly Gly Arg Ala Pro Ile Cys Leu Asp Glu Ser Ala
    775                 780                 785

cta ttt gag cag tac atg ccc ttc atg aaa ttg cag ttt gtc tat gca   2515
Leu Phe Glu Gln Tyr Met Pro Phe Met Lys Leu Gln Phe Val Tyr Ala
790                 795                 800                 805

cat cag gaa ggt ttt aaa gaa cag gga aca gaa gct cgt gaa ttt gga   2563
His Gln Glu Gly Phe Lys Glu Gln Gly Thr Glu Ala Arg Glu Phe Gly
                810                 815                 820

agt agc cgt ctt gtg aat ctt gcc tta cct atc ggg atc cga ttt gat   2611
Ser Ser Arg Leu Val Asn Leu Ala Leu Pro Ile Gly Ile Arg Phe Asp
            825                 830                 835

aag gaa tca gac tgc caa gat gca acg tac aat cta act ctt ggt tat   2659
Lys Glu Ser Asp Cys Gln Asp Ala Thr Tyr Asn Leu Thr Leu Gly Tyr
        840                 845                 850

act gtg gat ctt gtt cgt agt aac ccc gac tgt acg aca aca ctg cga   2707
Thr Val Asp Leu Val Arg Ser Asn Pro Asp Cys Thr Thr Thr Leu Arg
    855                 860                 865
```

FIG. 1H

att agc ggt gat tct tgg aaa acc ttc ggt acg aat ttg gca aga caa 2755
Ile Ser Gly Asp Ser Trp Lys Thr Phe Gly Thr Asn Leu Ala Arg Gln
870 875 880 885

gct tta gtc ctt cgt gca ggg aac cat ttt tgc ttt aac tca aat ttt 2803
Ala Leu Val Leu Arg Ala Gly Asn His Phe Cys Phe Asn Ser Asn Phe
890 895 900

gaa gcc ttt agc caa ttt tct ttt gaa ttg cgt ggg tca tct cgc aat 2851
Glu Ala Phe Ser Gln Phe Ser Phe Glu Leu Arg Gly Ser Ser Arg Asn
905 910 915

tac aat gta gac tta gga gca aaa tac caa ttc taa tgcgttagct 2897
Tyr Asn Val Asp Leu Gly Ala Lys Tyr Gln Phe
920 925

ttggtaaaga gctccataca tcgaagggaa aagagctttt aagatttctt gaaggctctt 2957

ttcgatttcg atttccattt tagtgttttg ctaaaacact ttc 3000

FIG. 1I

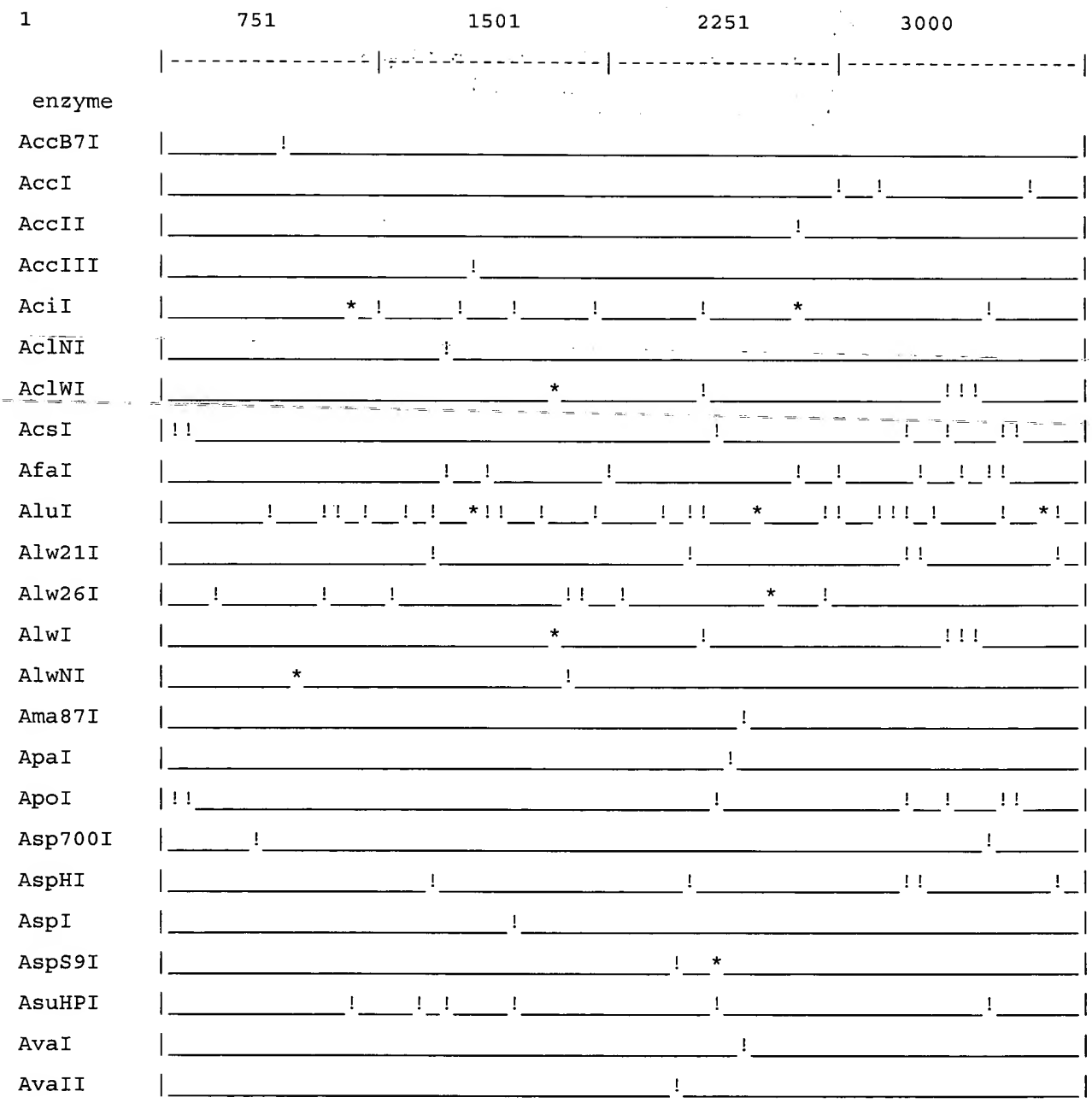
1
751
1501
2251
3000
enzyme
AccB7I
AccI
AccII
AccIII
AciI
AclNI
AclWI
AcsI
AfaI
AluI
Alw21I
Alw26I
AlwI
AlwNI
Ama87I
ApaI
ApoI
Asp700I
AspHI
AspI
AspS9I
AsuHPI
AvaI
AvaII

FIG. 2A

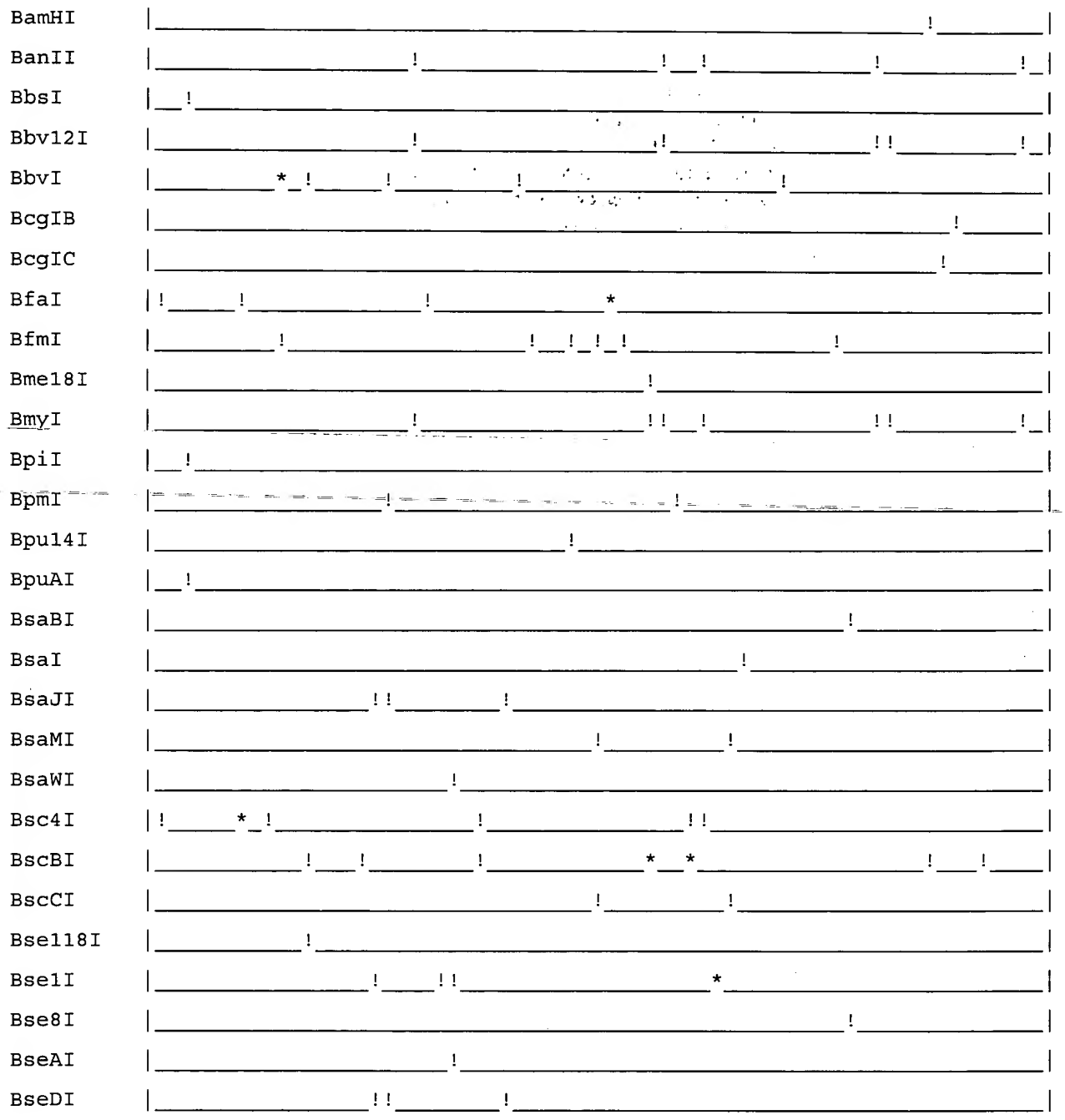
BamHI
BanII
BbsI
Bbv12I
BbvI
BcgIB
BcgIC
BfaI
BfmI
Bme18I
BmyI
BpiI
BpmI
Bpu14I
BpuAI
BsaBI
BsaI
BsaJI
BsaMI
BsaWI
Bsc4I
BscBI
BscCI
Bse118I
Bse1I
Bse8I
BseAI
BseDI

FIG. 2B

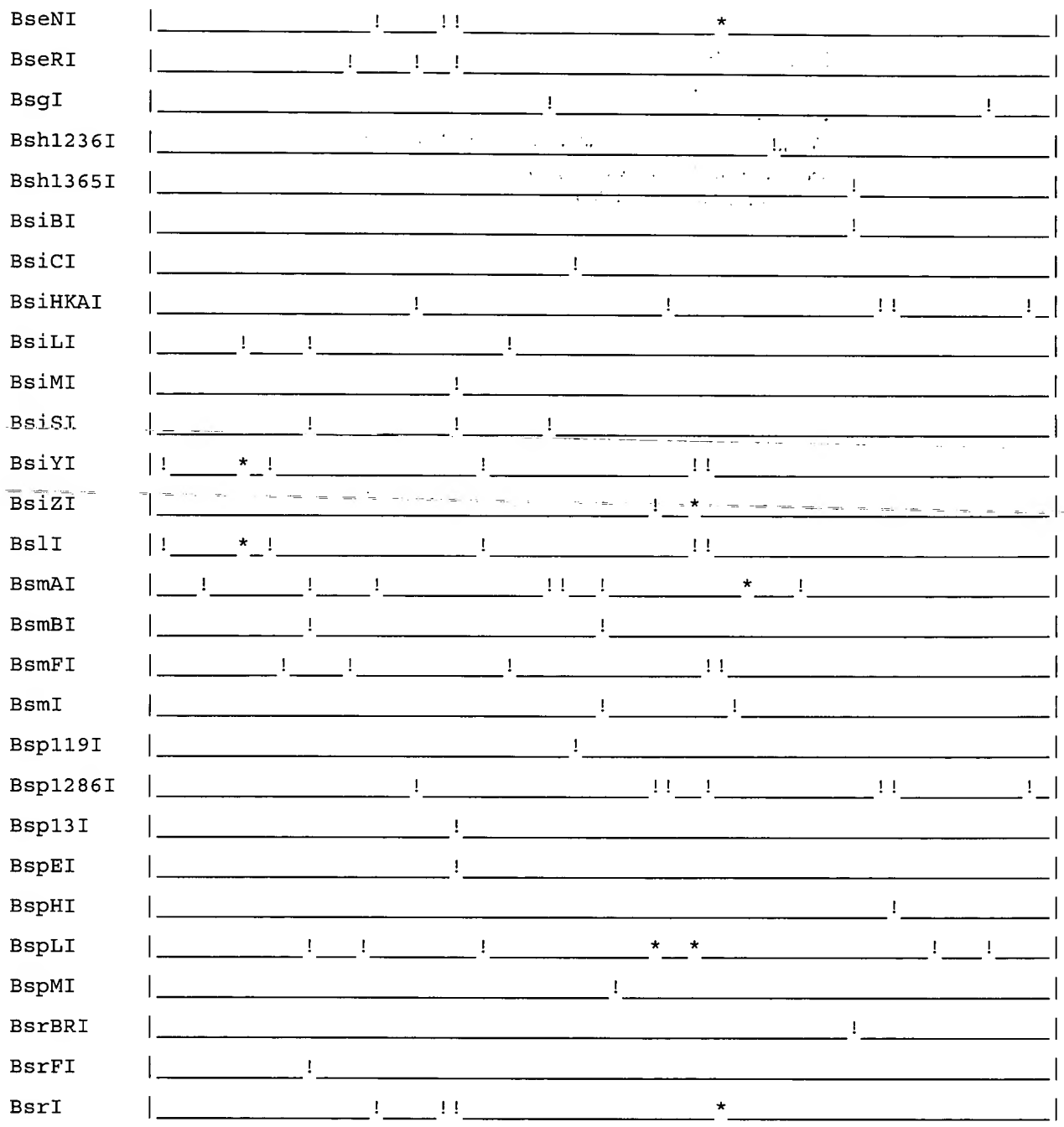
BseNI
BseRI
BsgI
Bsh1236I
Bsh1365I
BsiBI
BsiCI
BsiHKAI
BsiLI
BsiMI
BsiSI
BsiYI
BsiZI
BslI
BsmAI
BsmBI
BsmFI
BsmI
Bsp119I
Bsp1286I
Bsp13I
BspEI
BspHI
BspLI
BspMI
BsrBRI
BsrFI
BsrI

FIG. 2C

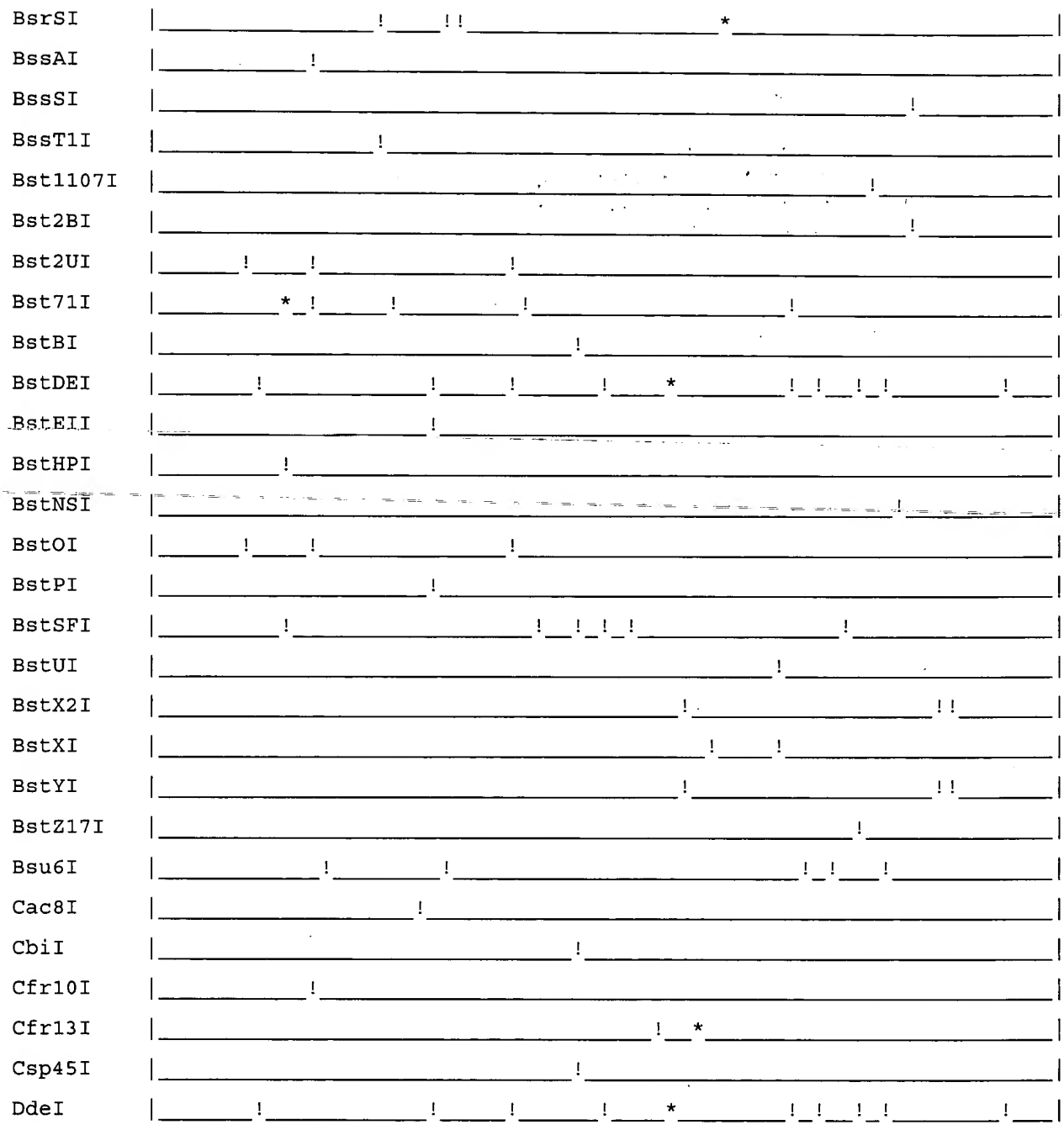
BsrSI
BssAI
BssSI
BssT1I
Bst1107I
Bst2BI
Bst2UI
Bst71I
BstBI
BstDEI
BstEII
BstHPI
BstNSI
BstOI
BstPI
BstSFI
BstUI
BstX2I
BstXI
BstYI
BstZ17I
Bsu6I
Cac8I
CbiI
Cfr10I
Cfr13I
Csp45I
DdeI

FIG. 2D

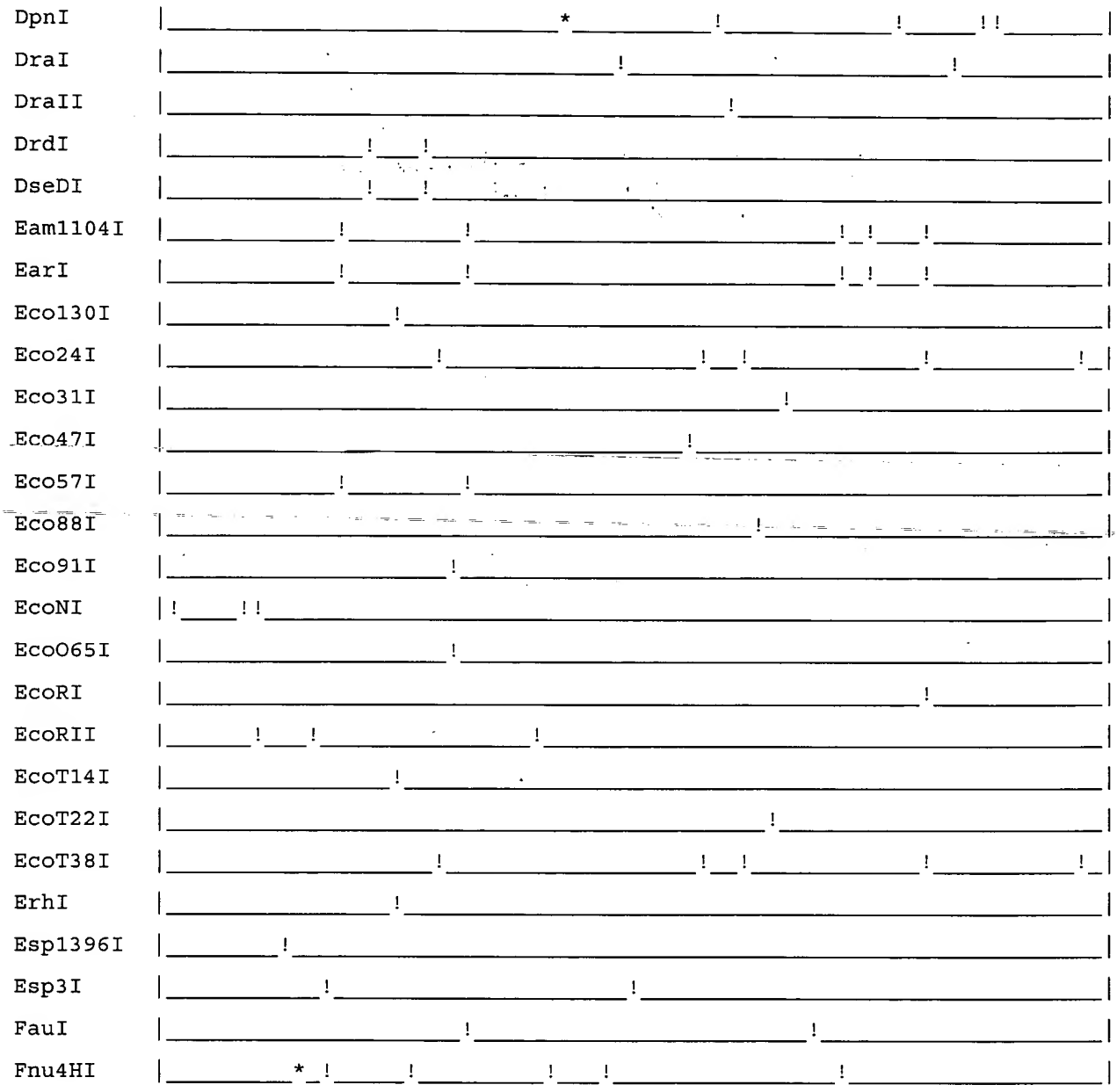
DpnI
DraI
DraII
DrdI
DseDI
Eam1104I
EarI
Eco130I
Eco24I
Eco31I
Eco47I
Eco57I
Eco88I
Eco91I
EcoNI
EcoO65I
EcoRI
EcoRII
EcoT14I
EcoT22I
EcoT38I
ErhI
Esp1396I
Esp3I
FauI
Fnu4HI

FIG. 2E

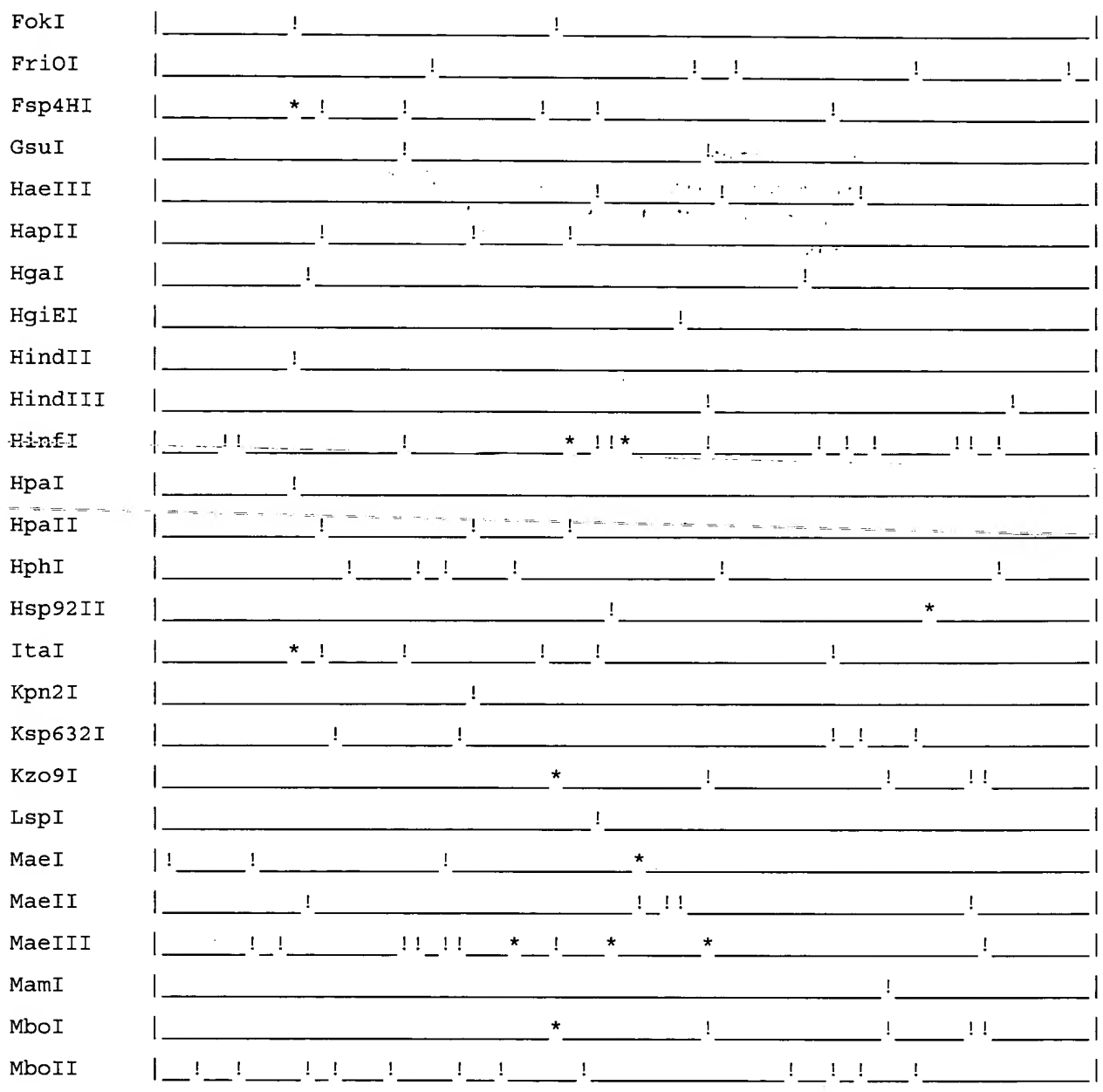
FokI
FriOI
Fsp4HI
GsuI
HaeIII
HapII
HgaI
HgiEI
HindII
HindIII
HinfI
HpaI
HpaII
HphI
Hsp92II
ItaI
Kpn2I
Ksp632I
Kzo9I
LspI
MaeI
MaeII
MaeIII
MamI
MboI
MboII

FIG. 2F

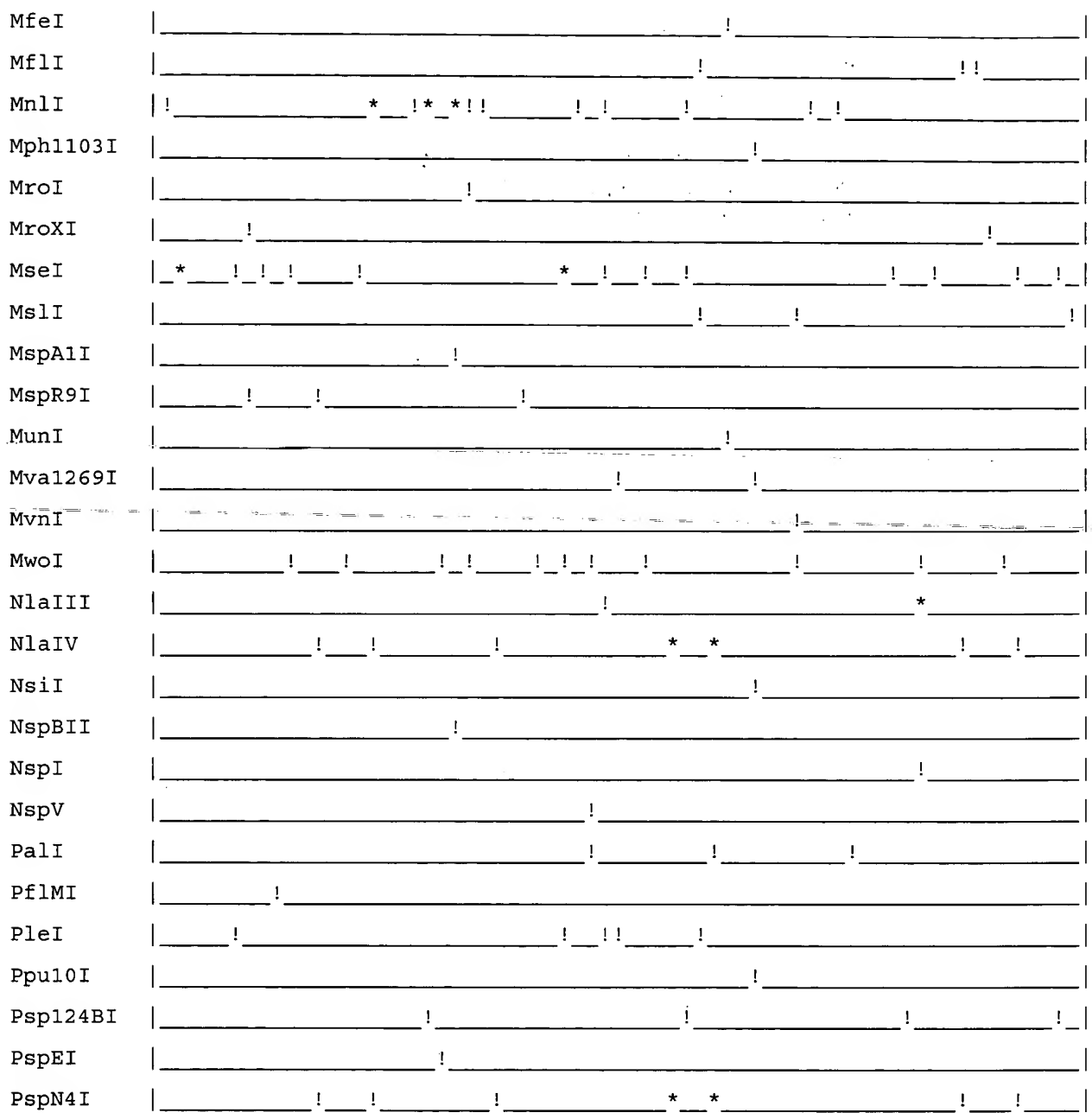
MfeI
MflI
MnlI
Mph1103I
MroI
MroXI
MseI
MslI
MspA1I
MspR9I
MunI
Mva1269I
MvnI
MwoI
NlaIII
NlaIV
NsiI
NspBII
NspI
NspV
PalI
PflMI
PleI
Ppu10I
Psp124BI
PspEI
PspN4I

FIG. 2G

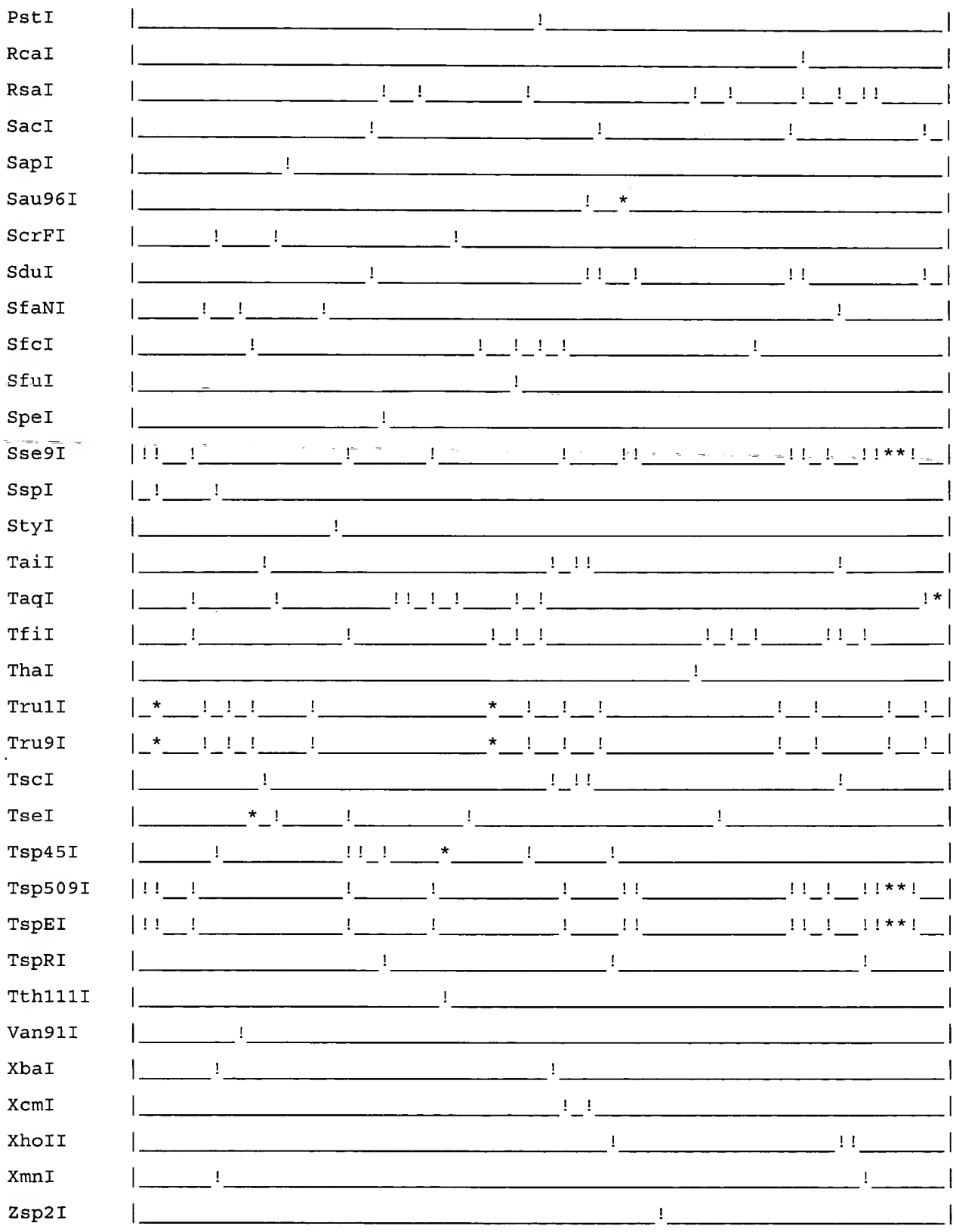
PstI
RcaI
RsaI
SacI
SapI
Sau96I
ScrFI
SduI
SfaNI
SfcI
SfuI
SpeI
Sse9I
SspI
StyI
TaiI
TaqI
TfiI
ThaI
Tru1I
Tru9I
TscI
TseI
Tsp45I
Tsp509I
TspEI
TspRI
Tth111I
Van91I
XbaI
XcmI
XhoII
XmnI
Zsp2I

FIG. 2H

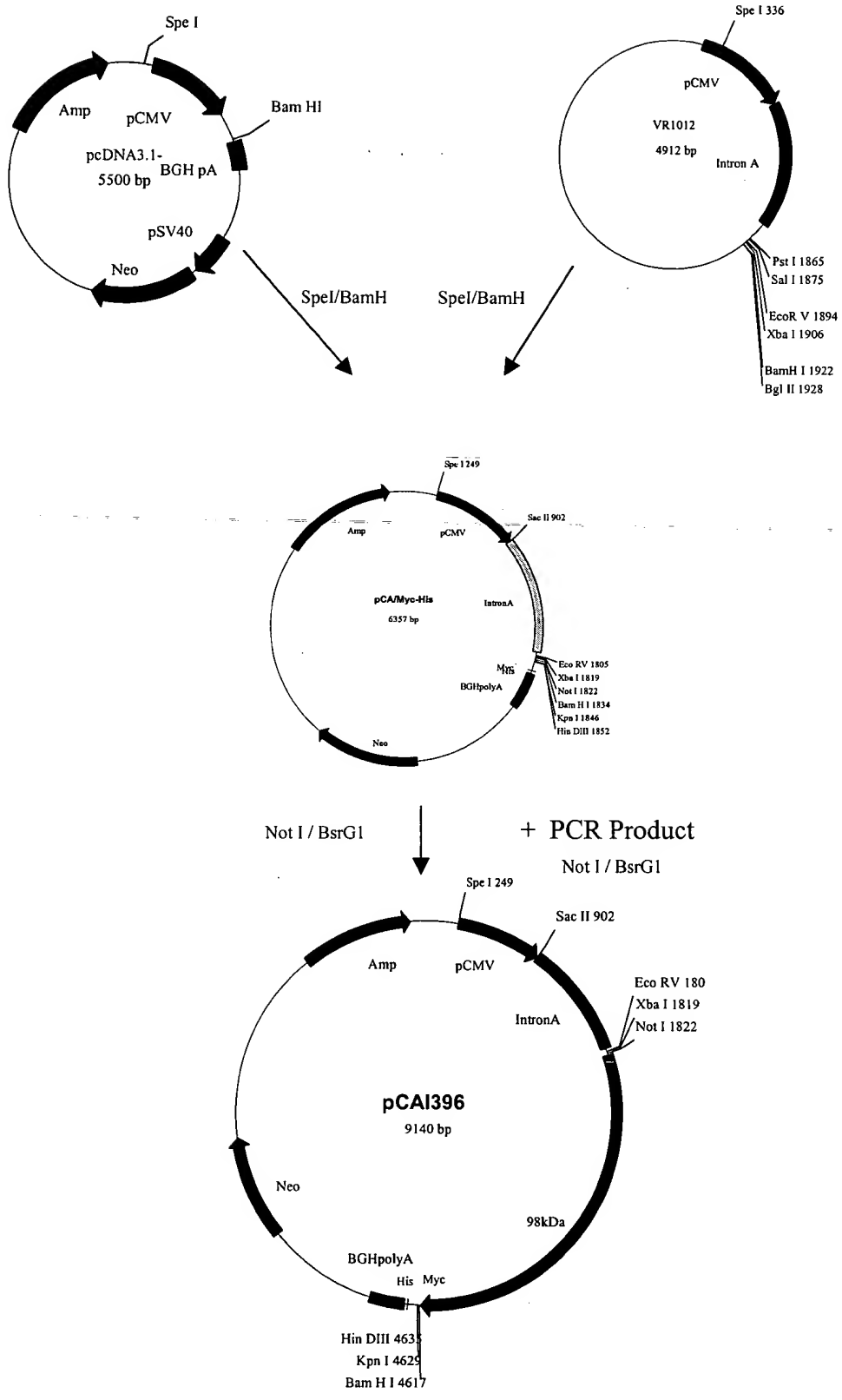
Spe I
Amp
pCMV
Bam HI
pcDNA3.1-
5500 bp
BGH pA
pSV40
Neo
SpeI/BamH
Spe I 336
pCMV
VR1012
4912 bp
Intron A
Pst I 1865
Sal I 1875
EcoR V 1894
Xba I 1906
BamH I 1922
Bgl II 1928
SpeI/BamH
Spe I 249
Sac II 902
Amp
pCMV
pCA/Myc-His
6357 bp
IntronA
Eco RV 1805
Xba I 1819
Not I 1822
Bam H I 1834
Kpn I 1846
Hin DIII 1852
BGHpolyA
Neo
Not I / BsrG1
+ PCR Product
Not I / BsrG1
Spe I 249
Sac II 902
Amp
pCMV
Eco RV 180
Xba I 1819
Not I 1822
IntronA
pCAI396
9140 bp
Neo
98kDa
BGHpolyA
His
Myc
Hin DIII 463
Kpn I 462
Bam H I 4617

FIG. 3

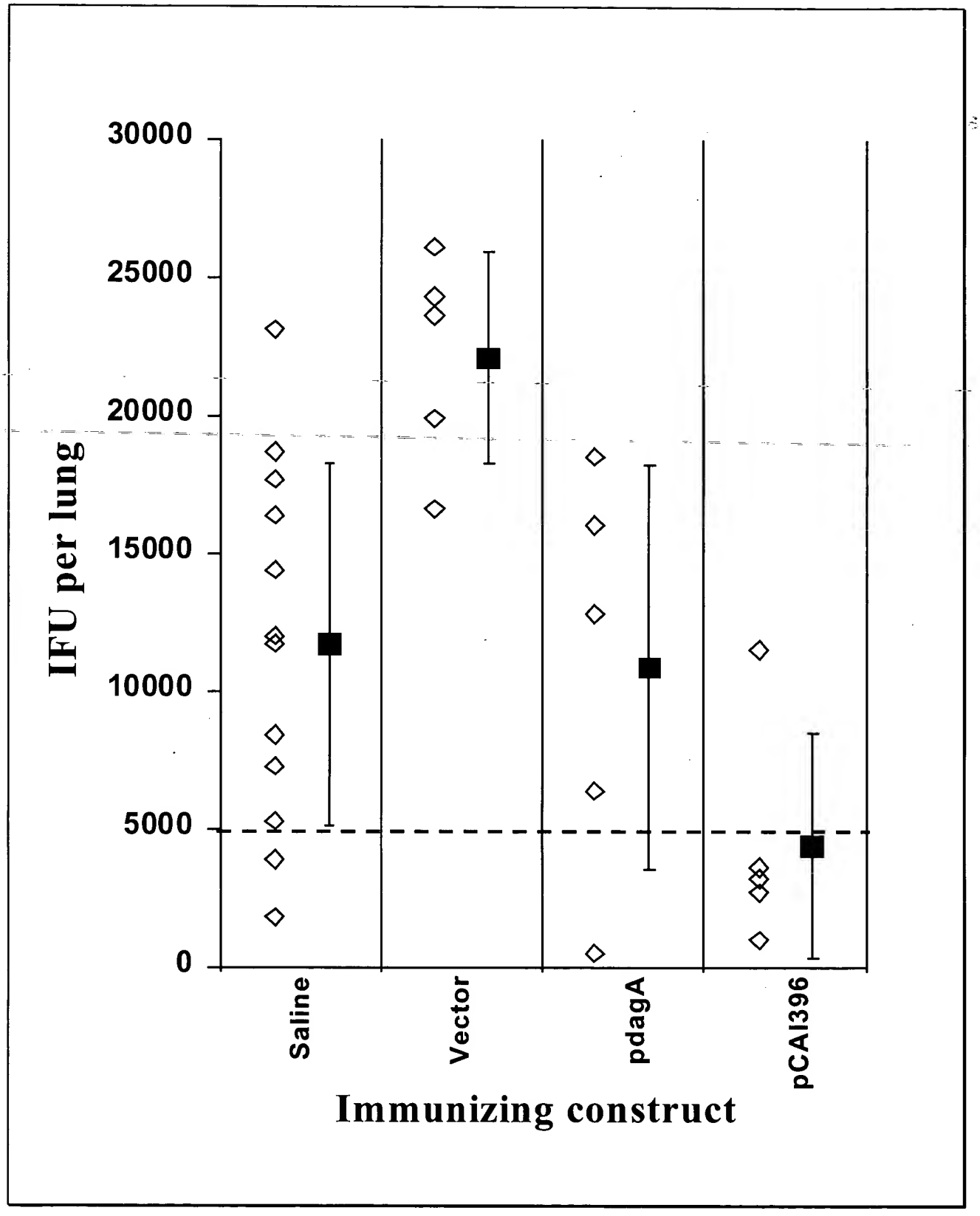
30000
25000
20000
15000
10000
5000
0
IFU per lung
Saline
Vector
pdagA
pCAI396
Immunizing construct

FIG. 4